

R 304 + - 3050

N053 - Mu Ab

(Book 20)

human iNOS Carboxyl Term: CR(NH)(am)-SLEMSAL/Thyre conjugate
4 shots @ 100µg/shot (1.6 mgm total)

- | | | |
|--|---|----------|
| 1) 1 st Immunization - IP | 0.8ml ag
0.8ml FcA
total 1.6 ml soln. | 10-11-93 |
| 2) 2 nd IP / FcA | SC/DW | 11-11-93 |
| 3) 3 rd IP / FcA | JC/RW | 12-2-93 |
| 4) Test Bleed by tail vein | IC/RW | 12-9-93 |
| 5) Test Bleed by tail vein | AF/RW | 12-30-93 |
| 6) 4 th IP/IV in PBS (Final pair to fusion)
R 3049 + R 3050 only | KF/RW | 1-4-93 |
| 7) R 3049 + 50 turned for fusion | IC/RW | 1-7-93 |
| 8) Test Bleed by tail vein | JC | 2-22-94 |
| 9) fused with fusion partners | | 3/7/94 |

EASY BEAM

Plate # 3 Date 12 / 14 / 93 Filter 492 nm
Operator SC Comment NO5.3 Test Bleed

Probe NOS4-rats
Plate sensitive @ 600 mg/l well

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.000	+OVER	+OVER	+1.450	+0.584	+0.132	+0.028	+0.000	+0.000	+0.000	+0.000	+0.000	A r 3047
B	-0.005	+OVER	+OVER	+OVER	+1.462	+0.543	+0.161	+0.060	+0.021	+0.008	+0.000	+0.000	B r 3048
C	+0.000	+OVER	+OVER	+OVER	+1.330	+0.441	+0.126	+0.040	+0.014	+0.000	+0.000	+0.000	C r 3049
D	+0.000	+OVER	+OVER	+OVER	+1.512	+0.536	+0.129	+0.033	+0.000	-0.005	+0.000	-0.006	D r 3050
E	+0.000	+OVER	+OVER	+1.972	+1.947	+1.813	+1.005	+1.268	+0.864	+0.482	+0.293	+0.173	E r 3047
F	+0.000	+OVER	+OVER	+OVER	+OVER	+OVER	+1.912	+1.779	+1.495	+1.128	+0.704	+0.375	F r 3048
G	+0.000	+OVER	+OVER	+OVER	+OVER	+OVER	+OVER	+1.880	+1.584	+1.260	+0.830	+0.530	G r 3049
H	+0.000	+OVER	+OVER	+OVER	+OVER	+OVER	+1.972	+1.890	+1.671	+1.428	+0.989	+0.621	H r 3050
		2	3	4	5	6	7	8	9	10	11	12	
		1-100	1-200	1-400	1-800	1-1600	1-3200	1-6400	1-12800	1-25600	1-51200	1-102400	

ISM

ISG

Black

EASY BEAM

Plate # 3
Operator Sc

Date 12 / 14 / 93
Comment N053 Test Bleed

Filter 492 nm

Probe N054-rats
Flat sensitize @ Konglwell

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.000	+OVER	+OVER	+1.450	+0.584	+0.132	+0.028	+0.000	+0.000	+0.000	+0.000	+0.000	A r3047
B	-0.005	+OVER	+OVER	+OVER	+1.462	+0.543	+0.161	+0.060	+0.021	+0.008	+0.000	+0.000	B r3048
C	+0.000	+OVER	+OVER	+OVER	+1.330	+0.441	+0.126	+0.040	+0.014	+0.000	+0.000	+0.000	C r3049
D	+0.000	+OVER	+OVER	+OVER	+1.512	+0.536	+0.129	+0.033	+0.000	-0.005	+0.000	-0.006	D r3050
E	+0.000	+OVER	+OVER	+1.972	+1.947	+1.813	+1.605	+1.268	+0.864	+0.482	+0.293	+0.173	E r3047
F	+0.000	+OVER	+OVER	+OVER	+OVER	+OVER	+1.912	+1.779	+1.495	+1.128	+0.704	+0.375	F r3048
G	+0.000	+OVER	+OVER	+OVER	+OVER	+OVER	+OVER	+1.880	+1.584	+1.260	+0.830	+0.530	G r3049
H	+0.000	+OVER	+OVER	+OVER	+OVER	+OVER	+1.972	+1.990	+1.671	+1.428	+0.989	+0.621	H r3050

I_{5M}

I_{5G}

1	2	3	4	5	6	7	8	9	10	11	12
Blank →	1 → 100	1 → 200	1 → 400	1 → 800	1 → 1600	1 → 3200	1 → 6400	1 → 12800	1 → 25600	1 → 51200	1 → 102400

Titers

I_{5M}:

- 1) r3047 - 700
- 2) r3048 - 1400
- 3) r3049 - 1400
- 4) r3050 - 1400

I_{5G}:

- 1) 47 - 9600
 - 48 - 26,000
 - 49 - 38,400
- 50 - 51200

EASY BEAM

Plate # 1 Date 12 / 31 / 93 Filter 492 nm
 Operator AF Comment N-054 50ng/well

test bleed

HRP-GAR IgG

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.000	+1.892	+1.851	+1.913	+1.925	+1.948	+1.861	+1.780	+1.596	+1.314	+0.949	+0.642	A rat#3047
B	+0.000	+1.960	+1.986	+OVER	+1.986	+1.925	+1.764	+1.607	+1.411	+1.001	+0.635	+0.359	B r3048
C	+0.000	+1.925	+1.960	+1.960	+1.960	+1.925	+1.902	+1.881	+1.712	+1.448	+1.117	+0.765	C r3049
D	+0.000	+1.960	+1.925	+1.960	+1.999	+1.925	+1.902	+1.772	+1.565	+1.244	+0.773	+0.473	D r3050

HRP-GAR IgM

E	+0.000	+OVER	+OVER	+OVER	+OVER	+1.290	+0.350	+0.085	+0.011	-0.010	-0.012	-0.014	E r3047
F	-0.012	+OVER	+OVER	+1.672	+0.756	+0.272	+0.101	+0.040	+0.009	-0.006	-0.010	-0.010	F r3048
G	-0.017	+OVER	+OVER	+OVER	+1.492	+0.648	+0.232	+0.075	+0.029	+0.000	+0.000	-0.007	G r3049
H	+0.000	+OVER	+OVER	+OVER	+OVER	+1.130	+0.363	+0.095	+0.015	+0.000	-0.006	+0.000	H r3050

1	2	3	4	5	6	7	8	9	10	11	12
Blank	1:100	1:200	1:400	1:800	1:1600	1:3200	1:6400	1:12800	1:25600	1:51200	1:102400

N053 Rat Fusion #2

Y3.1: 12xT-75: Counted 4xT-75

Pool #1 = 6.2×10^7 cells total @ 98% viability
 Pool #2 = 7.4×10^7 " " @ 100% "
 Pool #3 = 9.1×10^7 " " @ " "
 Pool #4 = 4.6×10^7 " " @ 97% "

Total = 2.73×10^8 @ viability avg = 98.8%

Y3.1 = 2.73×10^8 cells / 20 ml = 13.65×10^6 cells/ml

$$\begin{aligned} \#1 \quad & \frac{13.65 \times 10^6 \text{ cells}}{\text{ml}} = \frac{6.2 \times 10^7 \text{ cells}}{\text{ml}} \\ & = 4.5 \text{ ml} \end{aligned}$$

Need 4.5 ml Y3.1

$$\begin{aligned} \#2 \quad & \frac{13.65 \times 10^6 \text{ cells}}{\text{ml}} = \frac{7.4 \times 10^7 \text{ cells}}{\text{ml}} \\ & = 4.6 \text{ ml Y3.1} \end{aligned}$$

Fusion: Fused 2 spleens separately + plate out separately

Plating: Pool A = 5 x 96 well plates + 1 x 24 well

Pool B = 5 x 96 " " "

Saturday 8 Jan 1994 → FNO53 10x96 well + 1x24 well → fed 2 1 drop media

Fed N053 rat hybrids 2 1 drop/well of medium

IMDM + HAT

20% FCS

P/S

OPF

+ 4M NaOH

Sunday
1/9/94

{ Note fed 24 well - 6 drops of medium/well

EASY BEAM

 Plate # N053 #1A
 Operator JC/ KF

 Date 2 / 12 / 94
 Comment _____
Filter 492 nm

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.000	+0.000	+0.008	+0.006	+0.000	+0.000	+0.000	+0.000	+0.014	+0.000	+0.000	+0.000	A
B	+0.000	+0.000	+0.000	+0.107	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	B
C	+0.000	+0.000	+0.010	+0.000	+0.000	+0.000	+0.000	-0.006	+0.000	+0.000	-0.007	+0.012	C
D	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	D
E	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	-0.006	-0.006	+0.000	+0.000	+0.000	E
F	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	-0.006	+0.000	+0.000	+0.000	F
G	+0.000	+0.000	-0.005	+0.000	+0.000	-0.005	-0.005	-0.005	-0.007	-0.007	+0.000	+0.000	G
H	+0.000	+0.000	+0.008	+0.006	+0.000	+0.000	+0.043	+0.000	+0.000	+0.007	+0.000	+0.012	H
	1	2	3	4	5	6	7	8	9	10	11	12	

EASY BEAM

Plate # N053-3ADate 2 / 12 / 94Filter 492 nmOperator JO/KE

Comment _____

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.012	+0.009	+0.000	+0.000	+0.000	+0.000	+0.000	+0.011	+0.009	+0.000	+0.005	+0.012	A
B	+0.008	+0.008	+0.006	+0.000	+0.016	+0.000	+0.000	+0.000	+0.005	+0.009	+0.006	+0.000	B
C	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.011	C
D	+0.000	+0.008	+0.000	+0.000	+0.000	+0.012	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	D
E	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	-0.006	+0.000	+0.000	+0.000	+0.000	+0.000	E
F	+0.000	+0.005	+0.005	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.005	F
G	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	G
H	+0.000	+0.011	+0.009	+0.007	+0.000	+0.009	+0.000	+0.005	+0.000	+0.000	+0.000	+0.005	H
	1	2	3	4	5	6	7	8	9	10	11	12	

EASY BEAM

Plate # N053-4A Date 2 / 12 / 94 Filter 493 nm
 Operator JC/KE Comment _____

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.007	+0.007	+0.000	+0.007	+0.016	+0.010	+0.000	+0.000	+0.009	+0.009	+0.009	+0.009	A
B	+0.005	+0.000	+0.000	+0.000	+0.000	+0.007	+0.007	+0.012	+0.006	+0.009	+0.007	+0.014	B
C	+0.000	+0.005	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.005	+0.000	+0.014	C
D	+0.023	+0.008	+0.000	+0.000	+0.000	+0.000	+0.008	+0.000	+0.000	+0.000	+0.000	+0.019	D
E	+0.008	+0.000	+0.000	+0.000	+0.000	+0.000	+0.006	+0.000	+0.000	+0.000	+0.008	+0.034	E
F	+0.007	+0.000	+0.006	+0.000	+0.000	+0.005	+0.000	+0.006	+0.000	+0.010	+0.000	+0.023	F
G	+0.010	+0.000	+0.000	+0.000	+0.000	+0.000	+0.008	+0.013	+0.000	+0.000	+0.000	+0.022	G
H	+0.005	+0.008	+0.009	+0.006	+0.012	+0.008	+0.010	+0.010	+0.008	+0.005	+0.005	+0.017	H
	1	2	3	4	5	6	7	8	9	10	11	12	

EASY BEAM

Plate # N053 # 5A Date 2 / 12 / 94 Filter 492 nm
 Operator JC/ KF Comment _____

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.016	+0.005	+0.013	+0.000	+0.000	+0.000	+0.000	+0.000	+0.013	+0.013	+0.000	+0.021	A
B	+0.010	+0.010	+0.000	+0.000	+0.000	+0.008	+0.000	+0.000	+0.015	+0.000	+0.005	+0.005	B
C	+0.010	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	-0.006	+0.000	+0.005	C
D	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	D
E	+0.007	+0.005	+0.000	+0.000	+0.000	-0.006	+0.000	+0.000	+0.000	+0.000	-0.005	+0.009	E
F	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.008	F
G	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	-0.007	-0.005	-0.005	+0.000	+0.000	+0.017	G
H	+0.000	+0.000	+0.000	+0.000	+0.000	+0.012	+0.000	+0.000	+0.000	+0.000	+0.000	+0.019	H
	1	2	3	4	5	6	7	8	9	10	11	12	

EASY BEAM

Plate # N053-24 well (A)
Operator _____

Date 2 / 12 / 94
Comment _____

Filter 492 nm

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.038	+0.000	+0.000	+0.000	-0.005	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	A
B	+0.007	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	-0.005	+0.000	B
C	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	C
D	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	D
E	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	E
F	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	F
G	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	G
H	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	H
	1	2	3	4	5	6	7	8	9	10	11	12	

EASY BEAM

Plate # N053 #1b Date 2 / 12 / 24 Filter 492 nm
 Operator JC/KF Comment _____

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.009	+0.000	+0.000	+0.000	+0.000	+0.037	-0.006	-0.006	+0.000	+0.000	+0.000	+0.010	A
B	+0.000	+0.000	+0.000	-0.006	-0.006	+0.000	+0.000	-0.005	+0.000	+0.000	+0.000	+0.000	B
C	+0.000	+0.000	-0.005	+0.000	+0.000	-0.006	-0.006	-0.006	+0.000	-0.010	-0.006	+0.000	C
D	+0.000	+0.000	+0.000	+0.000	-0.006	-0.006	+0.000	+0.000	-0.005	-0.005	+0.000	+0.000	D
E	+0.000	+0.022	+0.000	+0.000	-0.007	+0.000	-0.007	+0.000	-0.007	-0.010	+0.000	+0.000	E
F	+0.000	+0.018	+0.000	+0.000	+0.000	-0.005	-0.005	-0.005	+0.016	-0.006	+0.000	+0.000	F
G	+0.000	-0.006	-0.006	-0.006	-0.006	+0.000	-0.007	-0.007	-0.005	-0.008	+0.000	-0.007	G
H	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	-0.007	+0.000	-0.007	+0.000	+0.000	H
	1	2	3	4	5	6	7	8	9	10	11	12	

EASY BEAM

Plate # N053-2B Date 2 / 12 / 94 Filter 492 nm
 Operator JC/kr Comment _____

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.009	+0.011	+0.008	+0.014	+0.006	+0.000	+0.000	+0.011	+0.000	+0.000	+0.000	+0.025	A
B	+0.000	+0.000	+0.008	+0.008	+0.008	+0.005	+0.009	+0.000	+0.000	-0.005	+0.000	+0.025	B
C	+0.000	+0.000	+0.000	+0.008	+0.000	-0.005	-0.005	<u>+0.186</u>	-0.005	-0.005	+0.043	+0.000	C
D	+0.000	+0.000	+0.000	+0.000	+0.000	+0.013	+0.005	+0.000	+0.000	-0.005	+0.000	+0.000	D
E	-0.005	-0.005	-0.005	-0.005	-0.008	-0.006	+0.015	+0.237	+0.000	-0.009	-0.006	-0.010	E
F	+0.000	-0.006	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	-0.010	-0.007	-0.005	-0.005	F
G	-0.005	-0.005	-0.008	+0.000	-0.006	-0.006	-0.005	-0.008	-0.008	-0.006	+0.000	-0.005	G
H	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	-0.005	+0.000	+0.000	+0.000	H
	1	2	3	4	5	6	7	8	9	10	11	12	

2BC8
2BE8

EASY BEAM

 Plate # N053 # 3B
 Operator XJ/KF

 Date 2 / 12 / 94
 Comment _____
Filter 492 nm

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.036	+0.014	+0.000	+0.010	+0.000	+0.005	+0.007	+0.007	+0.010	+0.000	+0.000	+0.016	A
B	+0.016	+0.006	+0.008	+0.011	+0.005	+0.044	+0.000	+0.019	+0.010	+0.007	+0.007	+0.011	B
C	+0.014	+0.000	+0.000	+0.007	+0.000	+0.000	+0.011	+0.000	+0.006	+0.000	+0.006	+0.008	C
D	+0.016	+0.006	+0.006	+0.000	+0.005	+0.013	+0.008	+0.006	+0.006	+0.013	+0.009	+0.009	D
E	+0.013	+0.000	+0.000	+0.045	+0.000	+0.005	+0.005	+0.008	+0.012	+0.006	+0.009	+0.011	E
F	+0.013	+0.005	+0.000	+0.006	+0.006	+0.000	+0.008	+0.011	+0.009	+0.015	+0.011	+0.011	F
G	+0.013	+0.000	+0.013	+0.000	+0.007	+0.000	+0.008	+0.005	+0.008	+0.008	+0.018	+0.011	G
H	+0.011	+0.008	+0.010	+0.015	+0.013	+0.009	+0.009	+0.011	+0.011	+0.014	+0.017	+0.017	H
	1	2	3	4	5	6	7	8	9	10	11	12	

EASY BEAM

Plate # N053 - 49 Date 2 / 12 / 94 Filter 492 nm
 Operator JC/KF Comment _____

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.000	+0.000	+0.000	+0.008	+0.008	+0.000	+0.000	+0.000	+0.005	+0.000	+0.016	+0.013	A
B	+0.005	+0.005	+0.000	+0.005	+0.000	+0.008	+0.000	+0.007	+0.007	+0.005	+0.007	+0.007	B
C	+0.000	+0.000	+0.000	+0.006	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.027	C
D	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.013	+0.008	+0.011	+0.000	+0.007	D
E	+0.000	+0.000	+0.031	+0.000	+0.000	+0.000	+0.000	+0.000	+0.014	+0.000	+0.000	+0.000	E
F	+0.000	+0.006	+0.000	+0.000	+0.000	+0.007	+0.000	+0.005	+0.000	+0.000	+0.000	+0.010	F
G	+0.008	+0.000	+0.000	+0.000	+0.005	+0.000	+0.000	+0.000	+0.000	+0.000	+0.009	+0.009	G
H	+0.011	+0.005	+0.009	+0.000	+0.005	+0.005	+0.007	+0.012	+0.012	+0.009	+0.009	+0.009	H
	1	2	3	4	5	6	7	8	9	10	11	12	

EASY BEAM

Plate # N053#58 Date 2 / 12 / 94 Filter 492 nm
 Operator JC/KF Comment _____

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.025	+0.014	+0.010	+0.016	+0.000	+0.005	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	A
B	+0.022	+0.005	+0.009	+0.005	+0.000	+0.000	+0.007	+0.005	+0.005	+0.000	+0.000	+0.022	B
C	+0.007	+0.000	+0.000	+0.005	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	C
D	+0.005	+0.000	+0.000	+0.005	+0.000	+0.000	+0.006	+0.000	+0.000	+0.000	+0.000	+0.000	D
E	+0.000	+0.000	+0.008	+0.000	+0.000	+0.033	+0.008	+0.000	+0.000	+0.000	+0.000	+0.008	E
F	+0.008	+0.000	+0.000	+0.000	+0.000	+0.000	+0.005	+0.000	+0.000	+0.000	+0.000	+0.010	F
G	+0.012	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	G
H	+0.000	+0.000	+0.000	<u>+0.060</u>	+0.000	+0.000	+0.005	+0.005	+0.009	+0.005	+0.000	+0.000	H
	1	2	3	4	5	6	7	8	9	10	11	12	

SBH4

EASY BEAM

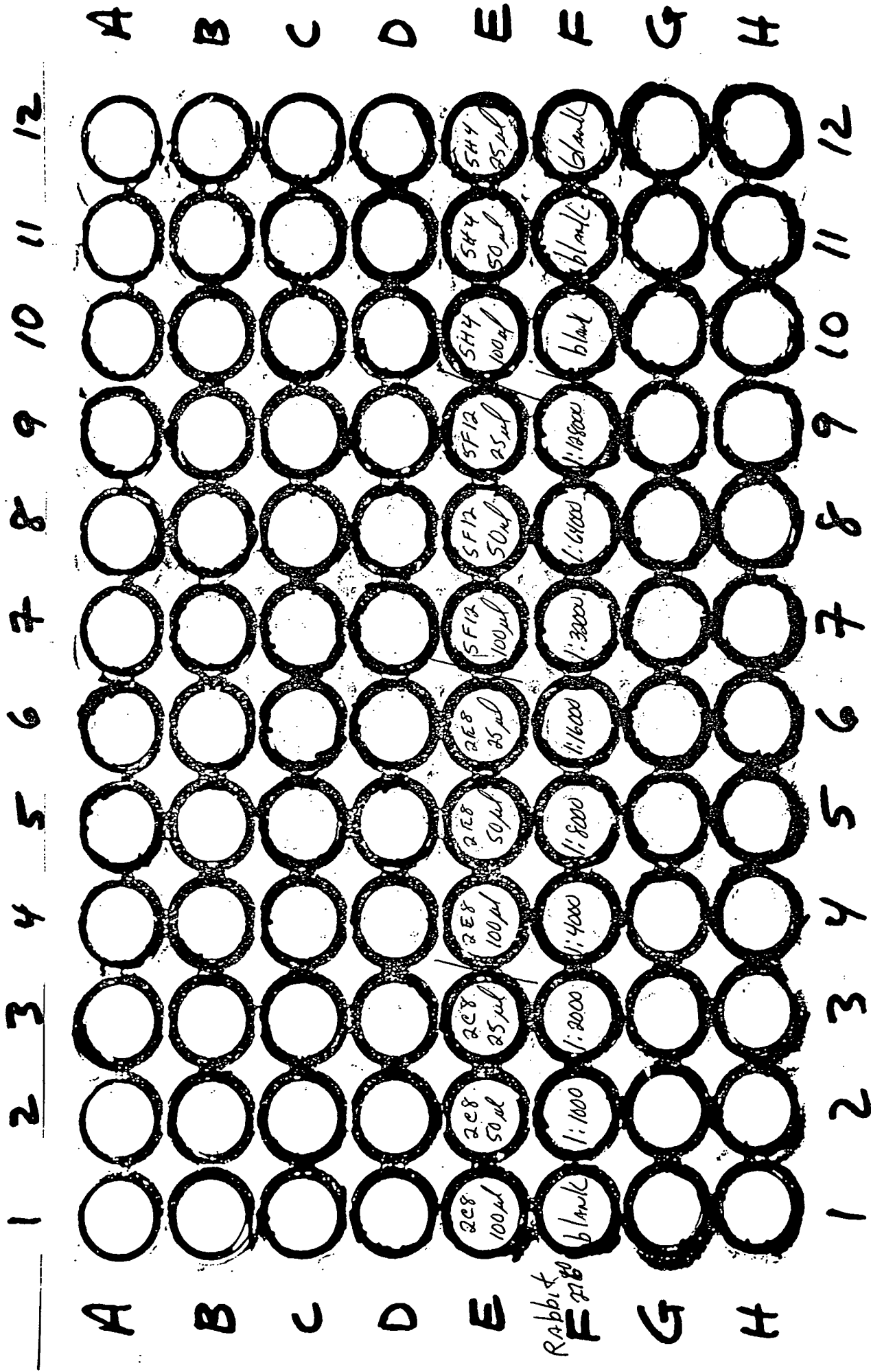
Plate # N053 Date 3 / 4 / 94 Filter 492 nm
 Operator KF Comment Sensitize @ 100ng/well N054
1° Ab = N053 culture supernatant

		1	2	3	4	5	6	7	8	9	10	11	12	
208	A	+0.013	+0.064	+0.020	+0.022	+0.015	+0.023	+0.023	+0.027	+0.034	+0.011	+0.011	+0.023	A
2E8	B	+0.021	+0.410	+0.227	+0.115	+0.084	+0.066	+0.041	+0.051	+0.044	+0.010	+0.044	+0.031	B
5F12	C	+0.010	+0.020	+0.022	+0.025	+0.025	+0.031	+0.026	+0.033	+0.036	+0.012	+0.012	+0.036	C
5H4	D	+0.020	+0.272	+0.139	+0.082	+0.059	+0.055	+0.039	+0.064	+0.041	+0.036	+0.020	+0.041	D
2C8	E	+0.000	+0.053	+0.021	+0.014	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.005	E
2E8	F	+0.000	+0.008	+0.008	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.005	F
5F12	G	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.005	+0.007	G
5H4	H	+0.000	+0.007	+0.007	+0.010	+0.005	+0.007	+0.007	+0.007	+0.000	+0.005	+0.005	+0.005	H
		1	2	3	4	5	6	7	8	9	10	11	12	
		Blank	172	174	178	1716	1732	1764	17128	17256	17512	171024	171048	

IgG

IgM

3-5-94



EASY BEAM

Plate # 1 Date 3, 6, 94 Filter 492 nm
 Operator DF Comment ADP Sephadex hi DWS (8-19/93) @ 10ng/well

	1	2	3	4	5	6	7	8	9	10	11	12	
A	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	A
B	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	B
C	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	C
D	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	D
E	100ul +0.000	2C8 50ul +0.000	85ul +0.000	100ul +0.009	2E8 50ul +0.000	25ul -0.009	100ul +0.000	60ul +0.006	25ul -0.005	100ul +0.000	5H4 50ul +0.000	25ul +0.000	E
F	blank +0.000	1:1000 +1.711	1:2000 +1.294	1:4000 +0.840	1:8000 +0.568	1:16K +0.307	1:32K +0.178	1:64K +0.058	1:128K +0.017	blank +0.006	blank +0.000	blank -0.006	F
G	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	G
H	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	H
	1	2	3	4	5	6	7	8	9	10	11	12	

NO Hybridoma Antibody
 binding to whole molecule

EASY BEAM

Plate # N053 Date 2 / 24 / 94 Filter 492 nm
 Operator JR Comment Sens. w/ N054 @ 100ng/well

		1	2	3	4	5	6	7	8	9	10	11	12	
r3047	A	+0.041	+OVER	+1.924	+1.771	+1.579	+1.255	+0.862	+0.497	+0.295	+0.137	+0.085	+0.058	A IgG
r3048	B	+0.033	+OVER	+1.998	+1.959	+1.860	+1.665	+1.458	+1.022	+0.669	+0.291	+0.169	+0.101	B
r3047	C	+0.000	+1.450	+0.419	+0.201	+0.112	+0.054	+0.029	+0.017	+0.008	+0.000	+0.008	+0.000	C IgM
r3048	D	+0.000	+1.912	+1.253	+0.739	+0.394	+0.185	+0.096	+0.116	+0.067	+0.059	+0.048	+0.025	D
	E	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	E
	F	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	F
	G	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	G
	H	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	H
		1	2	3	4	5	6	7	8	9	10	11	12	
		Blank	1:100	1:200	1:400	1:800	1:1600	1:3200	1:6400	1:12,800	1:25,600	1:51,200	1:102,400	

Rat NO53 Fusion (JR)V3.1

flask #1: attached: Total cells = 0.6×10^6 @ 97.2% viability
 floating: Total cells = 164×10^6 @ 97.6% viability

flask #2 + #3: floating: Total cells = 46.8×10^6 @ 94.2% viability

flask #4 + #5: floating: Total cells = 76.4×10^6 @ 92.3% viability

(flask #6 + #7: floating: Total cells = 57.9×10^6 @ 92.8% viability)

flask #8 + #9: floating: Total cells = 39.5×10^6 @ 95.7% viability

flask #10 \rightarrow : floating: Total cells = 14.3×10^6 @ 97.1% viability

Avg viability

Use all except flask 6 + 7:

Total cell count = 2.02×10^8

Avg viability = 97.1%

Splenocytes:

Total Avg cell count = 2.59×10^8 splenocytes

Fusion Ratio:

combine 1.3×10^6 Y3.i : \bar{c} splenocytes
 = 6.4 ml (out of 10 ml) Y3.i used \bar{c} splenocytes
 = 1.3×10^6 Y3.i : 2.6×10^8 splenocytes
 or: 2 splenocytes : 1 Y3.i

PEG added w/o loosening pellet \therefore slightly clumpy soln.
 after stirring firmly.

Plating: plate out cells onto 11 x 46 well plates at $\sim 40 \mu\text{l}/\text{well}$
 total volume of media + cells (*160 ml total cell soln.)

(KF) SP2/D-Ag14 12 x T-75: Add 10 ml fresh media

(JR) SP2/D-Ag14 12 x T-75: Add 10 ml fresh media

EASY BEAM

Plate # N053-1 Date 4 / 5 / 94 Filter 492 nm
 Operator JR Comment Sens w/ N054 @ Kong/well
2°Ab = HRP-GAr IgG/M cocktail

	1	2	3	4	5	6	7	8	9	10	11	12	
A	-0.005	+0.005	+0.000	+0.000	+0.000	+0.000	+0.000	-0.006	+0.000	-0.007	+0.008	+0.000	A
B	-0.006	+0.000	+0.000	+0.000	+0.005	+0.000	+0.000	+0.000	+0.005	+0.000	+0.086	+0.010	B
C	-0.008	+0.000	+0.005	+0.008	+0.015	+0.000	+0.009	+0.011	+0.006	+0.000	+0.006	+0.000	C
D	+0.000	+0.009	+0.016	+0.022	+0.005	+0.013	+0.013	+0.007	+0.000	+0.000	+0.016	+0.000	D
E	+0.000	+0.008	+0.012	+0.019	+0.000	+0.021	+0.012	+0.026	+0.019	+0.012	+0.012	+0.000	E
F	+0.005	+0.005	+0.007	+0.014	+0.017	+0.021	+0.012	+0.008	+0.010	+0.000	+0.022	+0.000	F
G	+0.000	-0.005	+0.000	+0.000	+0.008	+0.005	+0.000	+0.000	+0.000	+0.000	+0.000	-0.006	G
H	+0.000	+0.000	+0.000	+0.000	+0.006	+0.012	+0.006	+0.013	+0.000	+0.000	+0.012	+0.031	H
	1	2	3	4	5	6	7	8	9	10	11	12	

11311

EASY BEAM

Plate # N053-2 Date 4 / 5 / 94 Filter 492 nm
 Operator JR Comment Sens. w/ N054 @ 100ng/well
2°Ab = HRP-GAr IgG/M cocktail

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.000	+0.000	+0.000	+0.000	+0.000	+0.013	+0.000	+0.000	+0.014	+0.008	+0.012	+0.000	A
B	+0.005	+0.005	+0.008	+0.014	+0.011	+0.011	+0.016	+0.006	+0.010	+0.016	+0.007	+0.009	B
C	+0.000	+0.012	+0.012	+0.020	+0.023	+0.071	+0.024	+0.016	+0.036	+0.000	+0.017	+0.005	C
D	+0.000	+0.000	+0.008	+0.012	+0.019	+0.019	+0.014	+0.019	+0.013	+0.006	+0.009	+0.000	D
E	+0.000	+0.008	+0.031	+0.034	+0.024	+0.028	+0.028	+0.028	+0.016	+0.023	+0.006	+0.008	E
F	+0.000	+0.009	+0.015	+0.025	+0.014	+0.014	+0.024	+0.013	+0.023	+0.009	+0.009	+0.000	F
G	-0.005	+0.011	+0.006	+0.009	+0.005	+0.000	+0.006	+0.011	+0.000	+0.000	+0.008	-0.006	G
H	+0.000	+0.000	+0.015	+0.005	+0.011	+0.000	+0.012	+0.006	+0.013	+0.024	+0.007	+0.011	H
	1	2	3	4	5	6	7	8	9	10	11	12	

266

EASY BEAM

Plate # N053-3 Date 4 / 5 / 94 Filter 492 nm
 Operator JR Comment Sens. w/ N054 @ 100ng/well
2°Ab = HRP- GAR Ig G / M cocktail

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.007	-0.007	+0.000	+0.022	+0.000	+0.089	+0.000	+0.016	+0.000	+0.000	+0.000	+0.000	A
B	+0.011	+0.022	+0.000	+0.007	+0.010	+0.008	+0.016	+0.011	+0.007	+0.000	+0.000	+0.000	B
C	+0.000	+0.010	+0.013	+0.013	+0.016	+0.026	+0.009	+0.033	+0.027	-0.005	+0.015	+0.000	C
D	+0.006	+0.022	+0.016	+0.021	+0.006	+0.017	+0.023	+0.019	+0.019	+0.011	+0.011	+0.008	D
E	+0.023	+0.017	+0.017	+0.007	+0.019	+0.019	+0.016	+0.009	+0.024	+0.018	+0.000	-0.007	E
F	+0.008	+0.089	+0.019	+0.017	+0.017	+0.023	+0.013	+0.013	+0.006	+0.016	+0.006	+0.000	F
G	+0.000	+0.008	+0.000	+0.011	+0.000	+0.013	+0.005	+0.000	-0.009	+0.000	+0.005	+0.000	G
H	+0.000	+0.011	+0.011	+0.011	+0.007	+0.007	+0.005	+0.082	+0.000	+0.000	+0.000	+0.000	H
	1	2	3	4	5	6	7	8	9	10	11	12	

3A6
 3F2
 3H8

EASY BEAM

Plate # N053-4 Date 4 / 5 / 94 Filter 492 nm
 Operator JR Comment Sens w/ N054 @ 100ng/well
2°Ab = HRP-GAr IgG/M cocktail

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.000	+0.026	+0.010	+0.028	+0.007	-0.006	+0.018	+0.055	+0.034	+0.034	+0.008	+0.011	A
B	+0.024	+0.032	+0.010	+0.035	+0.035	+0.029	+0.044	+0.034	+0.063	+0.011	+0.057	+0.023	B
C	+0.035	+0.035	+0.010	+0.012	+0.056	+0.014	+0.042	+0.047	+0.049	+0.014	+0.048	+0.045	C
D	+0.018	+0.000	+0.036	+0.041	+0.024	+0.051	+0.036	+0.048	+0.021	+0.030	+0.030	+0.027	D
E	+0.019	+0.033	+0.009	+0.025	+0.032	+0.048	+0.051	+0.043	+0.046	+0.033	+0.061	+0.032	E
F	+0.010	+0.010	+0.025	+0.031	+0.006	+0.035	+0.031	+0.018	+0.028	+0.032	+0.021	+0.023	F
G	+0.005	+0.000	+0.046	+0.000	+0.038	+0.025	+0.015	+0.023	+0.026	+0.029	+0.000	+0.027	G
H	+0.005	+0.007	+0.019	+0.028	+0.022	+0.018	+0.044	+0.014	+0.057	+0.015	+0.018	+0.022	H
	1	2	3	4	5	6	7	8	9	10	11	12	

EASY BEAM

Plate # N053-5 Date 4 / 5 / 94 Filter 492 nm
 Operator JR Comment Sens. w/ N054 @ long/well
2°Ab = HRP-GAr Ig G/M cocktail

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.035	+0.015	+0.034	+0.015	+0.023	+0.036	+0.012	+0.010	+0.019	+0.015	+0.011	+0.007	A
B	+0.023	+0.031	+0.034	+0.045	+0.071	+0.025	+0.013	+0.032	+0.020	+0.011	+0.028	+0.009	B
C	+0.028	+0.018	+0.044	+0.024	+0.036	+0.025	+0.018	+0.018	+0.022	+0.027	+0.023	+0.016	C
D	+0.016	+0.012	+0.045	+0.033	+0.033	+0.012	+0.021	+0.021	+0.016	+0.027	+0.038	+0.016	D
E	+0.012	+0.028	+0.047	+0.019	+0.023	+0.023	+0.016	+0.022	+0.013	+0.036	+0.026	+0.016	E
F	+0.045	+0.028	+0.022	+0.015	+0.021	+0.016	+0.021	+0.026	+0.019	+0.017	+0.015	+0.017	F
G	+0.000	+0.021	+0.010	+0.030	+0.022	+0.020	+0.065	+0.026	+0.021	+0.047	+0.016	+0.009	G
H	+0.009	+0.018	+0.029	+0.031	+0.020	+0.031	+0.041	+0.051	+0.042	+0.032	+0.032	+0.017	H
	1	2	3	4	5	6	7	8	9	10	11	12	

SG7

EASY BEAM

Plate # N053-6 Date 4 / 5 / 94 Filter 492 nm
 Operator JR Comment Sens. w/ N05H @ 100 ng/well
2°Ab = HRP-GAr IgG / M cocktail

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.000	+0.000	+0.000	+0.007	+0.000	+0.014	+0.016	+0.067	+0.008	+0.008	+0.005	-0.015	A
B	+0.005	+0.020	+0.013	+0.018	+0.005	+0.010	+0.012	+0.016	+0.007	+0.013	+0.000	-0.018	B
C	+0.000	+0.000	+0.007	+0.005	+0.000	+0.013	+0.007	+0.000	+0.000	+0.015	+0.011	-0.022	C
D	+0.000	+0.005	+0.015	+0.012	+0.014	+0.014	+0.014	+0.012	+0.016	+0.023	+0.016	-0.019	D
E	+0.000	+0.013	+0.013	+0.013	+0.016	+0.021	+0.008	+0.018	+0.005	+0.000	+0.015	-0.019	E
F	-0.006	+0.005	+0.035	+0.015	+0.005	+0.033	+0.016	+0.020	+0.010	+0.012	+0.009	-0.019	F
G	+0.000	+0.005	+0.000	+0.010	+0.000	+0.021	+0.009	+0.009	+0.000	+0.000	+0.000	-0.020	G
H	+0.000	+0.012	+0.008	+0.019	+0.008	+0.010	+0.015	+0.009	+0.006	+0.006	+0.008	-0.018	H
	1	2	3	4	5	6	7	8	9	10	11	12	

GAS

EASY BEAM

Plate # N053-7 Date 4 / 5 / 94 Filter 492 nm
 Operator JR Comment Sens. w/ N064 @ Kong/Well
2°Ab = HRP-GAr IgG/M cocktail

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.000	+0.016	+0.025	+0.018	+0.021	+0.030	+0.007	+0.000	+0.031	+0.015	+0.034	+0.014	A
B	+0.000	+0.021	+0.021	+0.025	+0.038	+0.012	+0.016	+0.020	+0.020	+0.020	+0.017	+0.011	B
C	+0.015	+0.032	+0.011	+0.025	+0.018	+0.018	+0.033	+0.038	+0.025	+0.009	+0.019	+0.019	C
D	+0.017	+0.011	+0.026	+0.034	+0.036	+0.028	+0.028	+0.017	+0.020	+0.027	+0.036	+0.012	D
E	+0.032	+0.036	+0.013	+0.035	+0.024	+0.006	+0.013	+0.022	+0.022	+0.013	+0.031	+0.000	E
F	+0.020	+0.025	+0.025	+0.044	+0.026	+0.047	+0.020	+0.028	+0.023	+0.032	+0.015	+0.015	F
G	+0.000	+0.000	+0.015	+0.007	+0.015	+0.013	+0.007	+0.007	+0.000	+0.000	+0.018	+0.010	G
H	+0.007	+0.016	+0.018	+0.015	+0.015	+0.011	+0.018	+0.026	+0.017	+0.026	+0.017	+0.017	H
	1	2	3	4	5	6	7	8	9	10	11	12	

EASY BEAM

Plate # N053-B Date 4 / 5 / 94 Filter 492 nm
 Operator JR Comment Sens. w/ N054 @ 100ng/well
2°Ab=HRP-GA: IgG/M cocktail

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.000	+0.018	+0.009	+0.040	+0.028	+0.030	+0.021	+0.018	+0.080	+0.028	+0.017	+0.017	A
B	+0.013	+0.024	+0.043	+0.034	+0.028	+0.084	+0.052	+0.034	+0.025	+0.029	+0.034	+0.016	B
C	+0.014	+0.023	+0.038	+0.048	+0.028	+0.028	+0.030	+0.044	+0.051	+0.035	+0.029	+0.026	C
D	+0.016	+0.022	+0.047	+0.026	+0.029	+0.035	+0.041	+0.037	+0.041	+0.029	+0.022	+0.013	D
E	+0.013	+0.039	+0.014	+0.027	+0.011	+0.042	+0.025	+0.025	+0.025	+0.025	+0.010	+0.023	E
F	+0.017	+0.035	+0.020	+0.039	+0.044	+0.038	+0.042	+0.044	+0.023	+0.023	+0.043	+0.036	F
G	+0.011	+0.000	+0.018	+0.015	+0.020	+0.024	+0.008	+0.010	+0.024	+0.019	+0.000	+0.015	G
H	+0.000	+0.014	+0.014	+0.031	+0.031	+0.035	+0.000	+0.026	+0.013	+0.010	+0.000	+0.000	H
	1	2	3	4	5	6	7	8	9	10	11	12	

8A9

8B6

EASY BEAM

Plate # N053-9 Date 4 / 5 / 94 Filter 492 nm
Operator JR Comment Sens. w/ N054 @ 100ng/well
2°Ab = HRP-GArIgG/M cocktail

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.000	+0.000	+0.000	+0.005	+0.005	+0.005	+0.000	-0.006	+0.000	+0.000	+0.000	+0.000	A
B	+0.000	+0.008	+0.005	+0.009	+0.014	+0.014	+0.011	+0.009	+0.000	+0.009	+0.013	+0.005	B
C	+0.005	+0.007	+0.000	+0.012	+0.022	+0.017	+0.000	+0.011	+0.008	+0.000	+0.006	+0.009	C
D	+0.000	+0.008	+0.008	+0.014	+0.014	+0.014	+0.014	+0.005	+0.000	+0.009	+0.007	+0.000	D
E	+0.000	+0.000	+0.010	+0.000	+0.000	+0.000	+0.000	+0.005	+0.000	+0.005	+0.005	+0.000	E
F	+0.000	+0.000	+0.006	+0.006	+0.000	+0.000	+0.007	+0.013	+0.000	+0.005	+0.000	+0.000	F
G	+0.000	+0.000	+0.000	+0.007	+0.007	+0.007	+0.010	+0.006	+0.000	+0.000	+0.005	+0.000	G
H	+0.000	+0.017	+0.000	+0.016	+0.006	+0.006	+0.010	+0.006	+0.006	+0.000	+0.011	+0.007	H

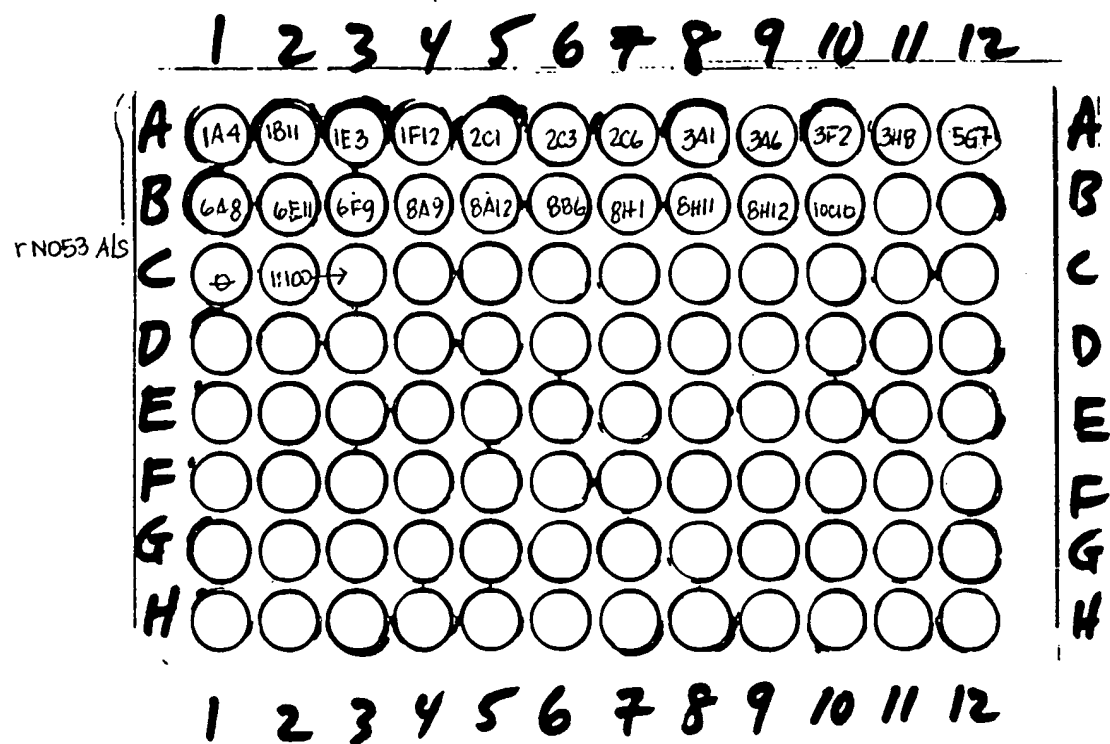
EASY BEAM

Plate # N053-10 Date 4 / 5 / 94 Filter 492 nm
 Operator JR Comment Sens. w/ N054 @ 100ng/well
2°Ab = HRP-GAr IgG/M cocktail

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.000	+0.000	+0.011	+0.000	+0.000	+0.000	+0.000	+0.000	+0.008	+0.008	+0.000	+0.000	A
B	+0.007	+0.010	+0.000	+0.010	+0.012	+0.000	+0.006	+0.000	+0.012	+0.007	+0.000	+0.000	B
C	+0.000	+0.000	+0.005	+0.010	+0.017	+0.007	+0.000	+0.000	+0.015	+0.011	+0.000	+0.000	C
D	+0.000	+0.005	+0.011	+0.007	+0.009	+0.009	+0.009	+0.007	+0.011	+0.013	+0.000	+0.000	D
E	+0.000	+0.000	+0.014	+0.005	+0.005	+0.005	+0.000	+0.000	+0.017	+0.012	-0.005	+0.000	E
F	+0.007	+0.005	+0.013	+0.021	+0.010	+0.007	+0.007	+0.012	+0.009	+0.012	+0.000	+0.000	F
G	-0.005	+0.000	+0.000	+0.000	+0.006	+0.000	+0.000	+0.000	+0.000	+0.000	-0.008	+0.000	G
H	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	H
	1	2	3	4	5	6	7	8	9	10	11	12	

Test Screen (100ul culture supernatant)

4/25/94



EASY BEAM

Plate # N053 Date 4 / 26 / 94 Filter 492 nm
 Operator KF Comment Sensitized @ 100ng/well

1°Ab = 100ul culture supernatant and Test Bleed A/S
2°Ab = HRP-GAR IgG/M cocktail

	1	2	3	4	5	6	7	8	9	10	11	12	
	1A4	1B11	1E3	1F12	2C1	2C3	2C6	3A1	3A6	3F2	3H8	5G7	
A	+0.000	-0.006	-0.028	+0.000	-0.006	-0.012	+0.030	+0.015	+0.015	+0.020	+0.090	+0.094	A
	6A8	6E11	6F9	8A9	8A12	8B6	8H1	8H11	8H12	10C10	Blank	Blank	
B	-0.012	-0.022	-0.016	+0.034	+0.050	+0.039	+0.000	-0.007	+0.020	+0.009	+0.000	+0.026	B
r N053	Blank	1→100											
A/S C	+0.000	+OVER	+1.936	+1.635	+1.508	+1.355	+0.884	+0.598	+0.344	+0.176	+0.071	+0.045	C
r 3047													
D	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	D
E	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	E
F	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	F
G	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	G
H	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	H
	1	2	3	4	5	6	7	8	9	10	11	12	

Microscopic Appearance:

3H8 - many round cells, appears very healthy

5G7 - fair number of round cells, some growing in colonies, appears very healthy

4-29-94



EASY BEAM

Plate # 3 Date 4, 30, 94 Filter 492 nm
 Operator DF Comment rhINOS (8-19-93 batch) @ 10 ng/well

100µl 50µl 25µl 100µl 50µl 25µl 100µl 50µl 25µl 100µl 50µl 25µl
 1 2 3 4 5 6 7 8 9 10 11 12

A	1A4	→	1B11	→	1E3	→	1F12	→				A
	+0.000	+0.000	+0.000	+0.012	-0.006	-0.006	-0.006	-0.006	-0.008	-0.010	-0.016	-0.016
B	2C1	→	2C3	→	2C6	→	3A1	→				B
	-0.007	-0.007	+0.014	+0.014	+0.000	-0.005	-0.005	-0.009	-0.011	-0.007	-0.005	-0.011
C	3A6	→	3F2	→	3H8	→	5G7	→				C
	-0.005	+0.000	+0.005	+0.000	+0.000	+0.010	-0.006	+0.000	+0.000	+0.005	+0.000	+0.000
D	6A8	→	6E11	→	6F9	→	8H11	→				D
	+0.000	+0.000	+0.000	-0.005	+0.000	+0.000	-0.009	+0.000	+0.000	-0.005	+0.000	+0.000
E	8A12	→	8B6	→	8H1	→	8H11	→				E
	+0.000	+0.000	+0.000	+0.000	-0.005	+0.010	-0.005	-0.007	-0.005	-0.008	+0.000	+0.000
F	8H12	→	10C10	→	blanks	→						F
	+0.000	+0.000	+0.000	+0.000	-0.005	-0.005	+0.000	-0.006	-0.006	+0.000	+0.000	-0.009
G	blanks	1:1000	1:2000	1:4000	1:8000	1:16K	1:32K	1:64K	1:128K	blanks		G
	+0.000	+0.772	+0.734	+0.599	+0.435	+0.314	+0.150	+0.066	+0.005	+0.000	+0.005	+0.000
H	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	H
	1	2	3	4	5	6	7	8	9	10	11	12

1Ab hit
276d

no binding to rhINOS!!

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